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Review

Findings on prenatal, lactational and later childhood exposure to dioxins and dioxin-like compounds: a review of the Amsterdam-Zaandam cohort 1987–2005

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Abstract: The Amsterdam-Zaandam cohort has been studied intermittently since 1987. The cohort was selected for optimal pregnancy and birth, in whom prenatal, lactational and more recently current dioxin exposures were measured. In the perinatal period and during the years thereafter, effects on various organ systems have been documented: thyroid, metabolism, immunity, haematology, motor development, brain development, lung function and puberty. We present a review of the endpoints studied, from the perinatal period into adolescence.

Keywords: long-term follow-up; dioxin exposure; PBDE; mother-child cohort

1. Introduction

In 1987 a study of the effects of prenatal and lactational dioxin exposure in children was initiated. This was the direct result of the discovery of dioxins in human breastmilk in the Netherlands and other Western European countries [1]. Exposure was relatively high (in comparison to today) and comparable to other Western European countries. Recruitment was initially slow but increased to 120 Mother-child pairs in 1990, following a successful grant application. The subjects

were residents of Amsterdam and the Zaandam region of the Netherlands. The study came to be known as the Amsterdam-Zaandam cohort. The mothers were included shortly before delivery. Inclusion criteria for the babies included: optimal pregnancy and birth, judged according to the then generally in use Prechtl score [2]; born at term; birthweight above 2500 grams. And all infants were to be exclusively breastfed until at least 11 weeks after birth. Informed consent was given by the parents. The mothers participating generally had a higher educational status, probably indicative of the inclusion criteria.

Originally the study period was from delivery until 6 months after birth. Later a follow-up was planned. Due to the inclusion criterium of at least 11 weeks of exclusively breastfeeding, the cohort decreased to a total of 60 (of the original 120 Mother-child pairs), of which 12 were included from 1987 to 1990, and the rest in the period 1990–1991. The children all met the inclusion criteria, and were exclusively breastfed during at least 11 weeks. The levels of dioxins and furans were measured in the breastmilk in the third week after birth. A psychomotor development test was performed shortly after birth, using the Prechtl method, and at the age of 6 months, using the Touwen neurological development test. At 7 days, 11 weeks and 6 months after birth, ultrasonography was performed to measure the size of the liver.

The following results were documented:

A diurnal variation in the dioxin levels was seen. The I-TEQ (international toxic equivalency value) levels in breastmilk of eight mothers were significantly higher in the evening than in the morning, but the difference was not large. In the morning a mean of 29.56 I-TEQ ng/kg fat and in the evening 31.60 I-TEQ ng/kg fat was measured. It was suggested that the energy intake during the day is low, and that in combination with the breastfeeding, the mobilisation of fatty acids plus dioxins took place from adipose tissue. It therefore seemed apparent that the time of day at which the breastmilk was sampled is also important with regards to the measurements. The pooling of samples taken at various moments during the day might be the most accurate manner of sampling, as was done [3].

The absorption of the pollutants by the baby was measured by quantification of the intake and faecal excretion of chlorinated dioxins (PCDDs) and dibenzofurans (PCDFs) in breastfed infants at 4, 8 and 12 weeks of age. The intake of PCDDs and PCDFs show a strong decline in the first 2 months when expressed in pg I-TEQ per kg bodyweight, mainly due to the growth of the baby. The intake, the concentration in the breastmilk multiplied by the amount of milk ingested, at 4 weeks after birth was 256.8, at 8 weeks 175.2 and at 12 weeks 120 pg ITEQ/kg bodyweight/day. Faecal excretion of the congeners was below five percent of the intake, indicating a bioavailability of more than 95% following breastfeeding. No obvious change was found in faecal excretion during these first 3 months of life [4].

In 41 healthy, well-nourished exclusively breastfeeding mothers, aged between 21 and 38 years, and their babies, several clinical and laboratory parameters were measured, aside from the dioxin content in breastmilk. The level of dioxins in the breastmilk was significantly related to the age of the mother. In the third week after delivery the women kept a food record for 7 consecutive days. There was an association between the daily intake of animal fats and proteins and dioxin concentrations in breastmilk fat, but not with vegetable fats, or carbohydrates [5].

Hereafter a study was performed, looking at the excretion of dioxins and dibenzofurans in the breastmilk of 34 healthy, well-nourished women participating in a special diet. In the fourth week after delivery, and after collecting data on her normal diet, the same mothers were randomly

prescribed either a low fat/high carbohydrate/low dioxin (20% energy from fat) during a week or a high fat/low carbohydrate/low dioxin diet (50% energy from fat). The percentage medium chain fatty acids (MCFAs) (C12:0 plus C14:0) was calculated as a measure for the de novo mammary synthesis of fatty acids, versus the percentage of the C18:19 long chain fatty acids (LCFAs) stored in adipose tissue. Although the mothers did not like the high fat diet, they followed the diet, as was seen by a significant change in the fatty acid composition of the breastmilk, as expected. The intake of dietary dioxin, measured in the breast milk of the mothers [4], was significantly reduced by both test diets. However the dioxin excretion in breastmilk/kg fat after one week of the diet was not significantly different from the levels before the diet. The concentration of dioxins in breastmilk seems to be independent of the source, either the adipose tissue or the de novo synthesis in the mammary gland. This finding suggests a rapid exchange of dioxins between different lipid compartments in the body [6].

1.1. Thyroid Hormone metabolism

In 38 Mother-child pairs thyroid function parameters were measured in the mother during delivery and in the baby in the cord blood, and at 7 days and 11 weeks after birth. Dioxin concentrations were measured three weeks after delivery and the infants were divided in a high and a low exposure group. The high exposure group had levels between 29.2–62.7 ng I-TEQ/kg milkfat (mean 37.5) and the low exposure group had levels between 8.7 and 28 ng I-TEQ/kg milkfat (mean 18.1). The levels of iodide in the urine were normal; there was no iodide deficiency in these babies. At birth there was a trend in the higher exposure group of a higher thyroxine (= Total T4) level and after 7 days T4 was significantly increased and after 11 weeks T4 was again significantly increased in combination with an increased thyreotropin (TSH). At 7 days and 11 weeks the T4/TBG (globulin) ratio was also significantly increased indicating that the thyroxin metabolism rather than the concentrations of the major thyroxin binding protein is affected. We concluded that increased concentrations of dioxins both intra-uterine and via breastmilk influence the pituitary-thyroid regulatory system. Cells in the hypothalamus were confronted with a hypothyroid state due to either a transport problem, or a lower activity of the 5′-deiodinase, or less T3 binding to the nuclear receptor, which was compensated by an increase in FT4 and also in TSH [7,8]. In a similar study, with similar exposure levels, in Rotterdam, lower FT4 was measured together with an increase in TSH [9]. This could indicate the activation of liver enzymes by dioxins, resulting in a higher excretion of thyroid hormone in the bile and faeces. We hypothesized a thyroid hormone resistance in our cohort, while the outcome of the study in Rotterdam was more indicative of a loss of thyroid hormone [7,9].

1.2. Growth, neurological development and liver size during the first 6 months of life

The neurological development, determined using the Prechtl score at 7 days, and the Touwen test at six months of age, showed no difference between the higher and lower exposed babies in our group [10]. Thus no neurological effect of the thyroid hormone dysregulation was evident. No effect was found on growth (weight, height and head circumference) or quetelet index during the first 6 months of life. Liver size and liver size/body weight ratio were not significantly different at 2 weeks and 11 weeks after birth, but there was a trend towards a smaller liver size at 2 weeks of age in the higher exposed group. This effect was no longer seen at eleven weeks after birth, indicating an

increased growth in the livers of the higher exposed group. At the age of 11 weeks two liver enzymes, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), were both increased in relation to a higher intake of dioxins via breastmilk [11].

1.3. Metabolism

Interestingly, the Retinol Binding Protein (RBP) levels were significantly lowered at eleven weeks of age in the higher lactational dioxin exposure group. This protein is produced by mature adipocytes and therefore lowering indicates a delay in the maturation of adipocytes in the first weeks after birth, probably caused by the dioxin exposure. This suggests an effect on energy metabolism.

The creatinine/bodyweight excretion in the urine was significantly lower in the higher prenatally dioxin exposed group both at eleven weeks and 26 weeks after birth. This might indicate an effect on body composition (less muscles and more fat), because there was no difference in bodyweight in the lower versus higher exposed group at these time points.

Another, as yet unexplained, finding was the significant lowering of the serum vitamin K levels in relation to the level of the congener tetra-chloro-dibenzofuran (TCDF) at eleven weeks postpartum. In our group at 11 weeks of age, a less significant lower Vitamin K1 level was also found related to the level of the congener Penta-CDD and Hepta-CDF. Furthermore an increase in proteins in absence of Vitamin K (PIVKA) related to Hx-CDF was seen [12]. Due to the administration of extra vitamin K to breastfed babies, only 8 subjects who received no suppletion could be evaluated. This possible TCDF effect on vitamin K levels is not an effect related to the TEQ level, and if true must be caused by the use of another pathway than the Aryl-hydrocarbon receptor. Most toxic effects of dioxins are mediated through the Aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor present in many cells.

1.4. Innate Immunity

Other clinical findings seen included a significantly lower number of polymorphonuclear neutrophils (PMNs) at 7 days of age in the prenatally higher exposed group (n = 18) and a lower number of thrombocytes at 11 weeks in the babies (n = 26) with a higher exposure via breastmilk. The findings of a negative influence of dioxins on the PMNs was confirmed in a Rotterdam study in 3 month old babies with similar exposure (n = 19) [13]. They also found a negative effect on the number of monocytes. All three forms of blood cells, the PMNs, thrombocytes and monocytes develop from the myeloid progenitor. The lymphocytes develop from the lymphoid progenitor and do not develop an Ah-receptor except under specific circumstances. At the age of 8 years (n = 27) a persistent negative effect on the thrombocytes was seen in relation to the lactational dioxin exposure. In addition, the thrombopoietin, a liver protein that regulates the number of thrombocytes, was inversely correlated to the thrombocyte count [14]. This indicates an influence at the stem cell level, or at the level of the myeloid progenitor cell, which is more likely because from this progenitor the above mentioned “dioxin sensitive” cells are formed. Thrombopoietin levels were also increased in relation to the lactational dioxin exposure [14]. This persistent effect was no longer found at the age of 15 years (n = 33) [15]. At that age a suppression of the PMNs was seen, related to a rather low WHO TEQ level of dioxin-like PCBs (77, 126, 169) in 33 adolescents. An animal study by Ackerman et al. supports the effect on the granulocytes (PMNs), but not on the “monocytic” (=

lymphoid) cells [16]. An effect on the number of thrombocytes was also found in Japanese workers with higher dioxin levels and in 2 Austrian women accidentally intoxicated with high concentrations of dioxins [17,18]. At follow-up of the Seveso cohort, an increased activity of the haemoglobin forming genes was found [19]. This seems logical since erythroblasts also develop from the myeloid progenitor cells.

2. Follow-up data

2.1. First follow-up at two and a half years of age

Various follow-up studies have been performed in the Amsterdam-Zaandam cohort. The first follow-up was performed when the children reached the age of 2.5 years. Only the subjects included in 1990/1991 were studied, because their laboratory data were complete. Thirty-eight subjects took part (16 boys and 22 girls). The prenatal exposure was 8.7–62.7 ng I-TEQ/kg milk fat, median 28.1. The group was divided into a higher and a lower exposure group (19 subjects in each group, 8 boys and 11 girls). The prenatal exposure was 8.7–28 ng I-TEQ/kg milk fat (mean 18.1) in the lower exposed group and 29.2–62.7 (mean 37.4) in the higher exposed group. All the babies were breastfed for at least 2 months, and most for longer. The cumulative dioxin intake was also divided into a lower and a higher exposure group (mean of 22.2, SD 13.7, ng I-TEQ dioxin and 67.7, SD 27.2 ng I-TEQ, respectively).

The Dutch version of the Bayley test was used to assess the neurodevelopment. No significant differences were found in mental (MDI-score) or psychomotor development (PDI-score), although the PDI-score was 9 points higher in the higher prenatally exposed group. The most surprising outcome was with the neurological examination performed according to the Hempel method. A lower score of sub-optimality, (meaning a better result) with a wider range of findings was seen in the higher exposure group and a lower reflex score (meaning higher reflexes) was seen in the subjects with higher prenatal exposure. In other words, an enhanced maturation was seen in the prenatally higher exposed group. The enhancement of the motor development might be explained by an agonistic effect of the perinatally higher T4 levels seen. As mentioned above, these higher levels were postulated as a compensation for the reduced hormone levels in the hypothalamus. This compensation might be necessary for certain tissues, like the hypothalamic cells. Yet, other tissues or parts of the brain might be stimulated, as was seen in tadpoles, with an enhanced metamorphosis caused by dioxins, and the enhanced development of teeth in the Yusho population [20,21]. At the age of two and a half years, the thyroid hormone levels were no longer different between the two groups, neither the prenatal nor lactational exposure. Additionally, the liver enzyme levels were no longer different. No blood smear or blood cell counts was performed during this follow-up [22].

2.2. Second follow-up at 7–12 years of age

2.2.1. Brain development

The second follow-up was performed at the mean age of 8 years, range 7–12 years. The complete cohort (n = 44) was included (including the original 12 Mother-child pairs from 1987–1989). The main aims of this follow-up included the evaluation of the intelligence and

psychological and neurophysiological outcomes. Other measured end points were lung function, thyroid function parameters, blood counts, liver enzymes and cytochrome P-450 activity.

It must be borne in mind, that only Mother-child pairs were included with an optimal pregnancy, birthweight and gestational age. Furthermore the average level of education of the mothers was significantly higher than the population norm. The results of the study on brain development in the 44 children showed a disparity in the outcomes between the usual psychological and neurological tests, performed using the Dutch version of the Revised Wechsler Intelligence Scale for Children (WISC-R), the Touwen test for neurological examination and the neurophysiological testing. Neither the psychological (WISC-R) nor the neurological (Touwen test) tests showed a difference between the higher and lower exposed children, neither with the prenatal nor with the lactational dioxin exposure. However in the neurophysiological tests, electro-encephalography (EEG) and evoked responses in combination with magneto-encephalography (MEG), significant differences in brain development were found. Longer reaction times were found both related to the higher prenatal and lactational exposures. Spontaneous alpha frequency and alpha amplitude were not affected. However, a 12 msec increased latency of the motion induced N2b was found, together with a higher amplitude. The latency of the N200 and the P3b components were also increased and the amplitude in the cognitive area was decreased, in relation to the higher exposures. This would indicate a negative influence on the reaction time due to a defective myelinisation and possibly a loss of neurons in the cognitive area of the brain. Obviously, these neurophysiologic testing methods are more sensitive than the psychological and neurological tests performed. With EEG and MEG a longer reaction time was measured of 12 milliseconds in the higher prenatally and lactationally dioxin exposed children. This is related to a normal reaction time of 140 milliseconds, meaning an almost 10 percent lower reaction time, indicative of a myelinisation problem. This was seen in relation to movements, but also when executing a cognitive task. In the cognitive area of the brain less neuron activity was found, as measured by the amplitude of the signal [23,24].

2.2.2. Behaviour

In 41 of the 43 children (2 were excluded) behaviour was evaluated using the Dutch version of the Teacher Report Form (TRF) for Children 4–18 years and the Child Behaviour Check List for Children (CBCL) 4–18 years. In the TRF more anxious/depressed, social and thought problems were seen and aggressive behaviour and a trend towards more attention problems, all related to lactational dioxin exposure. The CBCL showed more somatic complaints, anxious/depressed and social problems related to the prenatal dioxin exposure [24].

2.2.3. Innate and adaptive immunity

Effects on the immunity, both the innate and adaptive immunity were studied in venous blood at the age of 7–12 years. As described a persistent negative effect on the number of blood platelets, produced via the myeloid progenitor, were found, again related to the lactational dioxin exposure. In the adaptive immunity we also found effects related to the lactational dioxin exposure: more CD4 (T-helper) cells as well increased CD45RA cells. The T-cell/B-cell ratio was borderline-significantly raised in relation to the prenatal exposure. No other correlations were seen with the blood cells. No clinical indications of increased number of infections were seen in the cohort. Serum thrombopoietin

levels were measured. This protein is synthesised by the liver and regulates the number of thrombocytes in the circulating blood. Thrombopoietin was inversely correlated to the number of blood platelets, as expected, and positively correlated with the lactational dioxin exposures. We hypothesised that dioxins have a suppressive effect at the level of the stem cell or the myeloid progenitor cell [14]. We found this effect to be persistent until minimally eight years of age. Indeed, by the age of 15 years (n = 33) the effect of lactational dioxin exposure was no longer visible. However, at this later age a suppressive effect of the current dioxin-like PCBs on the number of PMNs was seen [15]. The serum levels of the WHO TEQ of the dioxin-like PCBs (congeners 77, 126, 169) were low compared to perinatal PCB levels found in The Netherlands in the years 1990–1991 [25]. The effect on the number of neutrophils during adolescence might be correlated to an increased bone marrow sensitivity to dioxin-like compounds, resulting from the perinatal exposure, a phenomena described after perinatal exposure to arsenic [26].

The effect on the thrombocyte counts is probably important in the perinatal period, since complications from thrombopenia include intracranial bleeding, the risk of which is increased should the neonate also have vitamin K deficiency. Vitamin K deficiency may be induced by a stimulation of liver enzymes by pollutants, as has frequently been documented in studies of anticonvulsants. Vitamin K levels in breastmilk are low. Severe gastro-intestinal bleeding was seen in Vietnam dioxin exposure (Agent Orange) victims. A combination of thrombocytopenia and hypoprothrombinemia was noted [27]. In our cohort, at 11 weeks of age a significantly lower Vitamin K1 level was found, related to the level of the congener TCDF, with a less significant relationship to Penta-CDD and Hepta-CDF. Furthermore, an increase in PIVKA was seen in relation to higher Hx-CDF exposure [28].

2.2.4. Liver

Effects on the liver were also studied in the cohort at the age of 7–12 years. A caffeine loading test was performed, to determine the Cyp 1A2 activity (n = 37). This activity was measured using the paraxanthine/caffeine ratio 6 hours after the caffeine loading. The histogram showed the typical slow and fast responders, known to be due to genetic predisposition. However, no correlation was seen with the prenatal or lactational dioxin exposures. We concluded that no long-term effect on the enzyme activity was seen [29].

Concurrently thyroid hormone activity was measured: TSH and free T4. No correlation with the prenatal or lactational exposures was seen at this age (n = 37). There was a negative correlation between the free T4 and the cytochrome P-450 1A2-activity. The liver enzymes ASAT and ALAT were within the normal range and no correlation was found with the prenatal or lactational dioxin exposures.

2.2.5. Lung function

Spirometry was performed in 41 children, 12 children had to be excluded, because of an unreliable measurement, but in the 29 children with reliable data, the prenatal and lactational dioxin exposures were related to more signs of obstruction using the FEV1/FVC ratio. The lung function was negatively influenced by both prenatal and even more so by the lactational exposure. After the Yusho, Yucheng and Seveso incidents, more respiratory problems were seen. Evidently, the lung is an organ sensitive to dioxin exposure [30]. The Clara cells have an Ah-receptor, and proliferate under

the influence of dioxins and secrete more mucous [30]. It would seem logical that they play a major role in the toxicity effects.

2.2.6. Dental development

In 41 children aged between 7 and 12 years the dental status was evaluated in relation to the prenatal and lactational dioxin exposures. No correlation was found between the exposures and the dental status in the cohort [31], contrary to a Finnish study wherein more dental problems were found to be related to lactational dioxin exposure [32]. Interestingly, in another study the open combustion of chemicals in the sixties in Amsterdam led to a sharp increase in non-syndromic orofacial clefts [33].

2.3. Third follow-up in adolescence at age 14–18 year

While the foetal and perinatal period are often referred to as the "first window of opportunity", puberty is often referred to as "the second window of opportunity", with respect to epigenetic changes. Follow-up during puberty included evaluating the long term effects of perinatal dioxin exposure into adolescence, but also the effects of current exposure to dioxins, dioxin-like PCBs (congeners 77, 126 and 169) and polybrominated diphenyl ethers (PBDEs), used as flame retardants. Once again development and growth, immunology and haematology, thyroid hormone metabolism, energy metabolism, lung function and behaviour were evaluated. The relatively high (Western European) background dioxin exposures of our cohort warranted investigating effects on and during puberty.

2.3.1. Exposure

The total number of subjects willing to participate in the follow-up during adolescence was 33, of which 14 girls and 19 boys [34]. Out of the 44 participants in the second follow-up 9 children could not be contacted, declined to participate or refused venepuncture, one child was excluded because he had developed an Ewing sarcoma, and one child had passed away because of leukaemia. The median prenatal dioxin exposure of the participants was 29.2 I-TEQ pg PCDD/Fs in the girls and 28.6 I-TEQ pg PCDD/Fs in the boys. Lactational dioxin exposure showed a median of 46 ng I-TEQ for the girls, and 42 ng I-TEQ for the boys. Current dioxin levels were determined. The current serum dioxin levels had a median of 1.1 WHO TEQ PCDD/Fs pg/g lipid in girls and 2.3 WHO TEQ pg/g lipid in boys. Range of the total group was 0.4–6.1 pg PCDD/Fs. The mean of the total group was 2.2 WHO TEQ PCDD/Fs and 95th percentile was 6.1 pg/g lipid serum.

The current serum levels of dioxin-like PCBs had a median of 1.7 WHO TEQ pg dl-PCBs in girls and a median of 1.5 WHO TEQ pg in boys. The range of the total group was 0.04–7.8 WHO TEQ pg/g lipid. The mean of the total group was 2.2 pg/g lipid and the 95th percentile was 7.3 pg/g lipid serum.

Current serum PBDE levels in ng/g lipid showed a median of 8.2 ng in girls (n = 9) and 9.5 ng in boys (n = 9). Mean of the whole group was 13.9 ng/g lipid serum, because of an outlier, range 4.9–73.6 ng/g lipid serum [34]. In the perinatal period these adolescents were not exposed to PBDEs. The brominated flame retardants began accumulating later in The Netherlands, after 1995.

2.3.2. Medical examination

A medical examination was performed, using the Tanner scale to determine the development of axillary and pubic hair, and breast and genital development.

One of the most important findings was a significant delay in onset of breast development in the girls (n = 14) correlated to both prenatal and lactational dioxin exposure. There was no correlation with the current serum dioxin levels. This finding of a delay in onset of puberty in girls is in agreement with an animal study. Furthermore, a Belgian study in 200 adolescent girls found a negative correlation between the current dioxin-like activity as measured with the Calux method and breast development [35,36,37]. Additionally, in the boys in our study, a delay in the first ejaculation was suggested, however not associated with the perinatal exposure, but with the current serum levels of dioxin-like-PCBs (n = 8) and with the sum of the current total TEQ of PCDD/Fs and dl-PCBs (n = 6). This last finding was not statistically significant, the number of subjects being small, but the scatter diagram shows a linear association. No correlations were found with either the perinatal exposure or the current levels with age at menarche of the girls.

The current axillary hair stage, testicular volume, penile length and current breast developmental stage were not related to the prenatal, lactational or the current dioxin levels. PBDEs could only be measured in 5 boys, due to logistical reasons, so no conclusions could be drawn.

2.3.3. Growth, weight, height and BMI

The height adjusted for age showed a positive correlation with the current serum PCDD/F WHO TEQ and this was also the case with the head circumference. No correlation was found between BMI, and weight with any of the exposures. In a Belgian study an increase in height in adolescents was seen in relation to dioxin-like activity measured with the Calux method [38].

2.3.4. Immunology and haematology

In the neonatal period clear negative effects were found on the number of PMNs and thrombocytes, as mentioned above, and the effect on the thrombocytes was persistent until at least 7–12 years after birth. The inverse correlation with the protein thrombopoietin indicated a problem at the level of the myeloid progenitor cells. Again the number of leucocytes and thrombocytes were counted and a differentiation of the blood cells was performed. In the adolescents (n = 33), no effect was seen on the number of leucocytes or on the number of PMNs, or other white cells, in relation to perinatal dioxin exposure. The number of thrombocytes and the thrombopoietin levels were also not correlated with the prenatal or lactational dioxin exposures. However, with the current level of WHO TEQ pg/g lipid in serum of dioxin-like PCBs (congeners 77, 126 and 169) a significant lowering of the PMNs was found. Thus, even at low levels (mean: 2.2 WHO TEQ pg/g lipid serum) of these dioxin-like PCBs a negative effect on the PMNs was detectable. Although the cohort size warrants caution in the interpretation of the outcomes, the fact that once again the PMNs are reduced, suggests that the finding is a legitimate one.

Adults seem to be less affected by an increase in infectious diseases in dioxin exposed cohorts. Epidemiology studies have also failed to provide convincing evidence that TCDD causes immune dysfunction in adults [39]. However in e-waste workers in Northern China an effect on innate

immunity (neutrophils) as a reactive oxygen species (ROS) alteration is described in 20 exposed workers at the age of 33 ± 3 years mainly related with the dioxin-like PCBs [40]. The number of neutrophils was increased contrary to our studies. It is not known if these workers were already perinatally exposed, but that is not likely considering the situation in China 30 years ago. Perinatal exposure of infants to dioxins or dioxin-like chemicals in 1990–1991 in The Netherlands, resulted in more otitis media and decreased incidence of allergies, consistent with suppression of immune function [41].

It seems logical that dioxins or dioxin-like compounds must bind and activate the Ah receptor in order to alter immune function. Thus studies of immunotoxicity should be performed in cells developed from the myeloid progenitor cell, since they develop de AH-receptor. However, studies are often performed in lymphocytes that develop from the lymphoid progenitor cell and in general do not develop the Ah-receptor. Only the lymphocyte Th17 cells have an Ah-receptor, but they form only a small percentage of the number of lymphocytes. TCDD suppresses the PMNs and these cells normally activate the lymphocytes. Yet, in most studies lymphocytes are studied. We found effects on PMNs at low levels of dioxin-like PCB congeners in adolescents. This is not a commonly reported finding, which might be explained by the choice of most researchers to examine effects on lymphocytes rather than PMNs. It is possible that an increased sensitivity to dioxin damage to PMNs later in life after perinatal exposure, has developed, comparable to arsenic toxicity [26]. It is also possible that even low levels of dioxin-like activity already have an effect on the myeloid progenitor cells of the bone marrow, independent of the perinatal dioxin exposure. However, an increase in the number of PMNs would then be expected, as found in the Chinese study. Our hypothesis can only be tested by studying a large cohort of adults born in Western Europe before 1970, since their perinatal exposure would be limited.

2.3.5. Thyroid hormone metabolism

We again measured serum levels of TSH, FT4, triiodothyronine (T3), antibodies against the thyroid system, and thyroxine-binding globulin (TBG) to study possible persistent effects or effects of current exposure. The current levels of T3 were positively correlated with serum BDE-99 levels and showed a positive trend with the sum of the PBDE congeners. There was a positive trend with the T4 levels. There was also a significant correlation between T3 and the current levels of dioxin-like PCBs. No correlations were found with the prenatal or lactational dioxin exposures and current TSH, T3, FT4 and TBG levels. Only one girl had measurable antibodies against thyroperoxidase (TPO).

It is disconcerting that we found significant correlations between thyroid hormone metabolism and both current BDE-99 levels (and a trend with the sum of the PBDEs) and with current levels of WHO TEQ dioxin-like PCBs [42]. Further research is warranted.

2.3.6. Insulin secretion

A clear negative effect of the prenatal and lactational dioxin exposure was found at the secretion of insulin in adolescence (n = 33). The effect of the prenatal exposure was also found at the glucose/insulin ratio. Fasting glucose levels were positively correlated with current WHO TEQ pg/g lipid of PCDD/Fs together with dioxin-like PCBs (= total TEQ). Once again an increased sensitivity,

triggered by the prenatal and lactational dioxin exposure, might explain these findings, even at the low current serum dioxin levels [43].

In a study of Seveso victims (n = 26) with high plasma dioxin level 20 years earlier, the HLA-DRB4 gene was down regulated, a gene that is, together with HLA-DR3, related to the development of type 1 diabetes. In the IGF-1 signalling pathway, insulin-like growth factor binding protein 7 (IGFBP7) was decreased, and genes related to carbohydrate metabolism were influenced [19].

Leptin levels were, as expected, highly correlated with BMI, as was the correlation between insulin and leptin. No significant results of exposure to either the prenatal or lactational PCDD/F exposures, or the current dioxin and dioxin-like PCBs and PBDEs were found in our group of 18 girls and 15 boys, in relation to leptin levels and BMI/leptin ratio. Leptin levels are strongly sex related. Neither was effects seen on the serum levels of cholesterol (high density lipoprotein and low density lipoprotein) and triglycerides, or with haemoglobin A1c levels.

The limited size of the follow-up cohort (n = 33) might play a role however the finding of lower insulin secretion, both related to the prenatal and lactational exposures, and the higher glucose/insulin ratio, seems a sound finding. Lower insulin secretion in relation to dioxin exposure has also been documented in animal studies [44,45]. The strong dose-response effect of persistent organic pollutants and development of diabetes is well known [46].

2.3.7. Behaviour

Thirty three adolescents were evaluated for behavioural problems, using Dutch versions of the CBCL 4–18 years and TRF 4–18 years questionnaires. In total 26 CBCL lists and 19 TRF lists were completed in the adolescence follow-up study. The prenatal and lactational exposures showed no significant correlations, but there was a positive trend between the lactational dioxin exposure and the total problem scores with the CBCL 4–18. The TRF showed no significant correlations with the prenatal and lactational exposures. However, with the current serum PCDD/F levels a positive trend was found with more externalising problems, and this was even more so with the cumulative current total TEQ PCDD/Fs and the dioxin-like PCBs [24].

The CBCL and TRF outcomes showed no correlation with the current serum PBDE levels.

2.3.8. Lung function

Lung function was also re-evaluated at puberty. A negative trend was found between prenatal dioxin exposure and FEV1 (p = 0.069) for the adolescent girls (n = 18). Animal studies have shown common environmental contaminant exposure to result in smaller numbers of alveoli and less branching of the pulmonary tree [47]. The number of alveoli and branching of the pulmonary tree probably do not increase after infancy [48,49]. In contrast to our earlier findings, there was no longer a correlation seen between FEV1/FVC and prenatal or lactational dioxin exposures in the complete follow-up cohort.

However, a positive correlation was seen between current serum PCDD/F levels and FEV1 (p = 0.038), and TLCO (p = 0.026) in girls/the girls seemed to have a better lung function. No associations were seen in the boys. For the dioxin-like PCBs no associations with lung function were found. Once again, the limited number of subjects in the adolescent follow-up may lead to wrong conclusions. Various major accidents, resulting in large populations being exposed to high

concentrations of dioxins, PCBs and furans have occurred. PCB, dioxin and furan exposure following rice oil contamination in Japan, in 1968, caused chronic bronchitis in 40% of the exposed and persistent suboptimal lung function. A similar incident on Taiwan Island led to 25% of the highly exposed babies dying within four years after birth as a result of respiratory disorders. Respiratory distress and pneumonia during the first six months of life were common [50]. The explosion at an Italian chemical plant led to high dioxin exposure, causing an increased mortality risk for respiratory disease, mainly chronic obstructive pulmonary disease (COPD). However, it must be borne in mind that, contrary to our study, these accidents were the result of an acute toxic effect with a high exposure level and not of a developmental effect.

A significant negative association was found between the sum of the BDE congeners and FEF 50 (p = 0.016). For BDE-100 and BDE-99 a significant negative association was found with FEV1/VCMAX (p = 0.031 and p = 0.049, respectively). This follow-up study suggests that brominated diphenyl ethers negatively affect lung function in teenagers. This observation suggests that at least a portion of the increased incidence of asthma throughout the Western World over the past decades may be the result of increasing BDE exposure. To our knowledge we are the first to link serum BDE levels to lung function deficits [51]. We measured current BDE levels. The perinatal levels are not known. However, in the years 1987–1991 the levels of brominated flame retardants in The Netherlands were very low. The exposure is predominantly from the last decade. It is alarming to find an association between BDEs and lung function. Polybrominated diphenyl ethers are very commonly used substances worldwide. Furthermore, indoor dust contains far higher concentrations than outdoor air. Recent studies have linked BDE exposures to endocrine disruption and neurotoxicity.

3. Conclusion

In this Mother-child cohort, selected for optimality and belonging to the higher social level in The Netherlands in the years 1987–1991, prenatal and lactational dioxin exposures were associated with effects on thyroid hormone metabolism and on the innate immunity in the neonatal period.

At 2.5 years, the thyroid hormone levels were normalised, but an enhanced motor development was seen.

In the primary school period (7–12 years of age), clear indications of negative effects on myelinisation of the brain were found related to the perinatal dioxin exposure, a delay of 12 milliseconds was seen in the higher exposed group. A persistent negative effect was seen of the early dioxin exposure on the thrombocytes and indications of damage at stem cell or myeloid progenitor cell level were found. An increase in pulmonary obstructive problems were found, associated with higher prenatal and lactational dioxin exposure, indicating long term effects of the early exposure.

At 12–18 years of age a clear negative association between start of breast development and higher perinatal dioxin exposure was seen. Again indications of effects of the early exposure on lung function were seen. The current serum levels of dioxin were very low, about tenfold lower than in the perinatal period. However, a negative effect on the innate immunity (fewer PMNs = granulocytes) in relation to the low level dioxin-like PCBs was seen, suggesting an increased sensitivity caused by the perinatal exposure. Again effects on thyroid hormone metabolism in association with current serum dioxin levels were seen, but also with current serum PBDE levels. Persistent effects of prenatal and lactational dioxin exposures were found in brain, lung, haematology, immunology and endocrine systems (pancreas and thyroid). Prenatal and lactational exposures, occurring during vulnerable

developmental windows, seem to have profounder effects than that following acute exposure in adulthood.

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Conflict of Interest

All authors declare no conflicts of interest in this paper.

Supplementary

Table S1. Publications of the Amsterdam-Zaandam cohort 1991–2014.

Primary author	Year	Number of subjects	Study focus	Outcome/conclusion	References
Koppe JG	1991	14	Coagulation effects in infants of dioxin exposure.	Possible relationship between dioxin exposure and haemorrhaging after birth.	[54]
Pluim HJ	1992	8	Diurnal variations in concentrations of dioxins and furans in human milk.	Concentrations significantly higher in the evening than in the morning.	[3]
Pluim HJ	1992	38	Dioxin effects on thyroid function in infants.	Higher mean T4, TSH and T4/TBG in higher exposed infants.	[7]
Pluim HJ	1993	3	Intake and faecal excretion of dioxins and furans in breast-fed infants at 4, 8 and 12 weeks.	Intake of dioxins and furans showed a strong decline during the first 3 months. Faecal excretion was < 5%, bioavailability > 95%.	[4]
Pluim HJ	1993	41	Relationship between dietary habits and dioxin and furan concentrations in human milk.	Strong correlation between levels in human milk and consumption of animal fats and proteins.	[5]
Pluim HJ	1994	34	Influence of short-term dietary measures on dioxin concentrations in human milk.	Short-term dietary interventions do not reduce dioxin concentrations in human milk.	[6]
Pluim HJ	1994	35	Clinical laboratory effects of dioxin exposure.	Reduced number polynuclear neutrophils in relation to higher dioxin exposure. Reduced thrombocyte count in relation to higher dioxin exposure. Increased liver transaminases in relation to dioxin intake.	[52]
Pluim HJ	1994	32	Relationship between dioxins and vitamin K status in infants.	Dioxins may play a role in vitamin K deficiency in infants.	[12]

Pluim HJ	1996	34	Relationship between dioxin and furan exposure and growth and development during the first six months of age.	No relationship was seen between exposure and growth and neurological development during the first six months of infancy.	[10]
Ilsen A	1996	32	Neurological development at 2.5 years of age in relation to prenatal and lactational dioxin exposure.	Psychomotor development (Bayley-test) and neuromotor functioning (Hempel-test) showed signs of enhanced maturation in relation to higher dioxin exposure.	[53]
Ten Tusscher GW	2001	29	Lung function in school children in relation to prenatal and lactational dioxin exposure.	Spirometry showed a negative association between Tiffenau index and both prenatal and lactational dioxin exposure.	[30]
Ten Tusscher GW	2003	27	Immunological and haematological effects in school children in relation to prenatal and lactational dioxin exposure.	Persistently decreased thrombocyte counts in relation to lactational exposure in infancy. Decreased allergy in relation to prenatal and lactational exposure. Increased counts of CD4+ T-helper and CD45RA cells.	[14]
Ten Tusscher GW	2008	37	Relationship between prenatal and lactational dioxin exposure and liver and thyroid parameters in school children	No persistent effects seen on thyroid hormones and liver transaminases. No association seen between the prenatal and lactational exposures and cytochrome P-450 activity.	[29]
Leijs MM	2008	33 (18 girls)	Effects of prenatal, lactational and current dioxin exposure on puberty.	A delay in initiation of breast development was found in girls with higher prenatal and lactational dioxin and furan exposure. Males revealed a negative trend with age at first ejaculation. No significant association was seen between the exposures and pubic hair growth, axillary hair growth, genital stage, height, BMI, testicular volume or menarche).	[36]
Leijs MM	2009	29	Effects of dioxins, PCBs, and PBDEs on immunology and	A decrease in the number of polymorphic neutrophils was found in adolescents with higher dl-PCBs in their serum	[15]

			haematology in adolescents.	(p = 0.021). No relation with total leukocytes, thrombocytes, hemoglobin, or thrombopoietin levels was seen. Similarly, we found no relation between prenatal, nor current dioxin levels and the hematological and the immunological parameters determined. The SigmaPBDEs were negatively associated with the number of lymphocytes (p = 0.01) and positively associated with the hemoglobin concentration (p = 0.003). These effects on the innate immunity by current levels of dl-PCBs and on the adaptive immunity by PBDEs are disconcerting, especially as the dl-PCB (0.04–7.8 WHOTEQ pg/g lipid, mean: 2.2 WHOTEQ pg/g lipid level was very low).	
Ten Tusscher GW	2014	41	Association between prenatal and lactational exposure and neurodevelopment at primary school age.	At prepubertal age no association was found between prenatal and lactational dioxin exposure and verbal, performal and total IQ or with the Touwen's test for neuromotor development. Behavioral problems associated with both prenatal and lactational dioxin exposure were found. Neurophysiological tests using magnetoencephalography and electroencephalography revealed an increase in latency time after a motion stimulus (N2b) of 13 ms (= a delay of 10%) in relation to higher prenatal dioxin exposure. A similar delay was measured in testing cognitive ability by analyzing the odd ball measurements, N200 and P300, together with an amplitude decrease of 12%. The delay is indicative of a defective myelinisation and the decrease in amplitude of a loss of neurons.	[24]

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